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PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

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PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Rule 71.1)

Date of mailing *(day/month/year)*	19.10.2000
Applicant's or agent's file reference PBA/D088165PWO	IMPORTANT NOTIFICATION

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/GB99/01691	14/06/1999	12/06/1998

Applicant
CENTRAL MANCHESTER HEALTHCARE NHS TRUST et al.

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

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Form PCT/IPEA/416 (July 1992)

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
PBA/D088165PWO		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/GB99/01691	14/06/1999	12/06/1998

International Patent Classification (IPC) or national classification and IPC
C12Q1/68

Applicant
CENTRAL MANCHESTER HEALTHCARE NHS TRUST et al.

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 7 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 3 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☒ Certain documents cited
   - VII ☒ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
11/01/2000	19.10.2000

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Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/GB99/01691

I. Basis of the report

1. This report has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.*):

Description, pages:

1-27 as originally filed

Claims, No.:

1-11 with telefax of 18/09/2000

Drawings, sheets:

1/9-9/9 as originally filed

2. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

3. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

see separate sheet

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/GB99/01691

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-11
	No:	Claims	-
Inventive step (IS)	Yes:	Claims	-
	No:	Claims	1-11
Industrial applicability (IA)	Yes:	Claims	1-11
	No:	Claims	-

2. Citations and explanations

see separate sheet

VI. Certain documents cited

1. Certain published documents (Rule 70.10)

and / or

2. Non-written disclosures (Rule 70.9)

see separate sheet

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:

see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

INTERNATIONAL PRELIMINARY EXAMINATION REPORT - SEPARATE SHEET

International application No. PCT/GB99/01691

Section I:

The Sequence Listings (SEQ ID NOs 1-39) filed on 15.02.2000 do not form part of the application (Rule 13ter 1(f) PCT).

Section V:

The following documents are referred to in this communication:

D1: WO 92 15678 A
D2: NEWTON ET AL: 'PCR, 2. Auflage' 1997 , SPEKTRUM AKADEMISCHER VERLAG , HEIDELBERG
D3: EP-A-0 613 945
D4: WO 99 16904 A (8. April 1999)

D1, which is discussed in the application (appl.: p 2 para 5- p 3 para 4), describes a method for generating dicistronic DNA molecules (Fig. 4). In this method first stage PCRs are carried out concurrently in one reaction vessel (p 3; claim 2). In the second stage PCR, the fusion PCR, the generated fragments are joined (p 21). In some embodiments the outside primers are used in excess relative to the inside primers, which correspond to the "primers with linker sequences" of the present application (D1: p 49 l 19- p 50 l 21; claims 4, 5, 14). During the amplification process, the inside primers are exhausted within about 2 to about 12 thermocycles (p 50 l 17-21). In other words, in the method of D1 the inside primers are present in limiting concentrations.

1 Novelty (Art 33(2)PCT):

1.1 **Claim 1** is novel, because none of the available documents discloses a method of producing a hybrid DNA molecule combining three or more fragments, which embraces first stage PCRs carried out in a single reaction mixture and a second stage PCR in which the amplified fragments are joined.
For the same reason also dependent **claims 2-9** and **11** are novel.

1.2 **Claim 10** is novel, because the primer sequences have previously not been disclosed.

2 Inventive Step (Art 33(3)PCT):

2.1 **Claim 1** does not appear to satisfy Art 33(3)PCT for the following reasons:
D1, which is considered to represent the closest prior art, is distinguished from the subject-matter of claim 1 in the following:

1. D1 only discloses the fusion of two DNA fragments.
2. D1 does not take into account that some DNA polymerases viz Taq polymerase add a 3' adenosine overhang to an extended strand.

ad 1. The extension of the method of D1 to a method which involves the fusion of three of more DNA fragments seems to be a simple straightforward modification.

ad 2.
The problem to be solved in claim 1 appears to be the provision of a more efficient method for the fusion of the amplified fragments (application: p 2 para 3).
In order to solve the problem, the skilled person would turn to D2, as D2 reviews methods for the fusion of overlapping PCR products (p 79-84). It is pointed out, that Taq polymerase adds an adenosine to the 3'-end of an extended fragment and that this has to be taken into account when designing primers for amplified fragments (p 81 para 1; Fig. 4.8). The primers shown in D1 (Fig. 4.8) were designed with a different strategy than the primers of the present application. According to D1, primers are selected which anneal to sequences on the template which are adjacent to a T nucleotide. According to the present application primers are designed which contain an additional A nucleotide on their 5' end. The selection of either design and the related advantages and restrictions appear to be obvious for the skilled person once he knows about the polymerase property to add an a nucleotide to the 3' end of the extended product.
Thus, the method of D1 in combination with the general teaching of D2 that some DNA polymerases add an A to the extended product renders the solution of **claim 1** obvious. The same argument applies to **claims 2-5, 7-8** and **11** which relate to simple modifications of the method of claim 1 and therefore do not contain an inventive concept per se.
Claim 6 relates to a method which involves freezing of the reaction mixture after the first stage PCR in order to deactivate "residual PCR activity". First of all, PCR

is a reaction and thus does not have any activity. By freezing the reaction mixture the polymerase is deactivated and the reaction is stopped. Thus, apparently "residual PCR activity" is supposed to mean "residual polymerase activity". However, when the mixture is thawed for the second stage PCR, the polymerase is reactivated and involved in the second PCR stage.
In other words, deactivation of polymerase after the first stage PCR does not seem to solve a problem and can thus not be considered inventive (Art 33(3) PCT).
Claim 9 relates to a method of mutation analysis, which is effected on a hybrid DNA molecule produced by the method of any of claims 1-8. Methods of mutation analysis are known in the art (e.g. D3: claim 17) and since claim 9 does not contain any technical feature in addition to the method features of claims 1-8, it does not appear to be inventive either.

2.2 **Claim 10** does not appear to satisfy Art 33(3)PCT.
The sequence of NF2 gene and its exon structure is known from D3 (Seq ID No 15; Table 1). The selection of primers for the amplification of fragments of the NF2 gene appears to be commonplace in the art (see for example D3: Table 2) and there is no indication found throughout the application that the chosen primers achieve an unexpected effect. Above all, it is mentioned in the application, that the primers were designed with the help of a commercially available computer program (application: p 14 para 6). In other words, the primers are the result of a known commonplace design method. The addition of an adenosine residue at the 3'-end of each primer appears to be obvious in view of the characteristics of DNA polymerases such as Taq polymerase and the use of the primer for amplified fragments (see also point V 2.1 above). Hence, the set of primers of **claim 10** does not appear to involve an inventive concept.

Section VI:

This written opinion is based on the assumption that all claims enjoy priority rights from the filing date of the priority document (12.06.1998). If it later turns out that this is not correct, the document D4 (publication date: 08.04.1999) could become relevant to assess whether the subject-matter claimed satisfies the criteria set forth in Article 33(1)PCT. Moreover, in some cases D4 may become relevant in the regional phase

(e.g. before the EPO (Art 54 (3) EPC)) for the assessment of novelty.

Section VII:

Contrary to the requirements of Rule 5.1(a)(ii) PCT, the relevant background art disclosed in the documents D2 and D3 is not mentioned in the description, nor are these documents identified therein.

Section VIII:

Claim 1 does not meet the requirement of clarity (Art 6 PCT), because there appears to be an error in step (3). From the description it is understood, that in the second stage PCR a pair of primers is used one of the primers providing the 5'-end of the sense strand and the other providing the 5'- end of the antisense strand (description: p 9 para 4). The examination of the claim has been carried out on the basis of the description.

Claim 2 does not satisfy Art 6 PCT because its dependency is not clear. A claim cannot be dependent on itself.

CLAIMS

1. A method of producing a hybrid DNA molecule having a sense strand and an anti-sense strand and in which, reading in the 5' to 3' direction, the sense strand has the sequences x_1, x_2.....x_n, where n is greater than or equal to 3, the method comprising the steps of

(1) providing in a single reaction mixture

(a) the sequences x_1, x_2.....x_n and their complementary sequences x_1', x_2'.....x_n', to be assembled into the hybrid molecule,

(b) for each pair of complementary sequences defined in (a) a respective pair of PCR primers each having a priming sequence and which are such that the primers for the 3' ends of any two sequences (x_i, $x'_{(i+1)}$), where i is 1 to (n-1), have specifically complementary linker sequences connected to their respective priming sequences via an adenine residue, and

(2) effecting a first stage PCR reaction in which those primers provided with linker sequences are present in limiting concentrations and a polymerase which adds a 3' adenine overhang to the end of an extended strand; and

(3) effecting a second stage PCR reaction using a single pair of primers one of which provides the 5'-end of the sense strand and other of which provides the 3'-end of the anti-sense strand of the required hybrid molecule

whereby said hybrid molecule is generated.

2. A method as claimed in claim 2 wherein the polymerising enzyme for steps (2) and (3) is *Taq*.

3. A method as claimed in claims 1 or 2 wherein the annealing temperature (T_m) of the linker sequences is greater than that of the priming sequences to the x and x' sequences.

4. A method as claimed in claim 3 wherein the annealing temperature of the linker sequences is 2 to 5°C greater than that of the priming sequences to the x and x' sequences.

5. A method as claimed in any one of claims 1 to 4 wherein the linker sequences do not have intrinsic secondary structure.

6. A method as claimed in any one of claims 1 to 5 wherein between the first and second stage PCR reactions the reaction mixture is frozen to deactivate residual PCR activity.

7. A method as claimed in any one of claims 1 to 5 wherein between the first and second stage PCR reactions the reaction mixture is treated with an exonuclease I to digest single stranded molecules.

8. A method as claimed in any one of claims 1 to 7 wherein each of the first and second stage PCR reactions utilise a thermally activated polymerase.

9. A method of mutation analysis wherein the analysis is effected on a DNA hybrid molecule produced in accordance with the method of any one of claims 1 to 8.

10. A set of primers incorporating the following sequences.

5'tcatcmagccgctgcattgcc-a-3'

5'ggccatgcagcggctaatatga-a- 3'

5'agccactacccaaactcctgt-a-3'

5'acaggtgttgggtagtggct-a-3'

5'tgtctcactgaacctgcctacct--a-3'
5'aggtaggcaggttcagtgagaca-a-3'

5'cctcat taccggctgtcagactg-a-3'
5'cagtctgacagccggtaatgagg-a-3'

11. A method of producing a hybrid DNA molecule having a sense strand and an anti-sense strand and in which, reading in the 5' to 3' direction, the sense strand has the sequences x_1, x_2.....x_n, where n is greater than or equal to 3, the method comprising the steps of

(1) providing in a single reaction mixture

(a) the sequences x_1, x_2.....x_n and their complementary sequences x_1', x_2'.....x_n', to be assembled into the hybrid molecule,

(b) for each pair of complementary sequences defined in (a) a respective pair of PCR primers each having a priming sequence and which are such that the primers for the 3' ends of any two sequences (x_i, $x'_{(i+1)}$), where i is 1 to (n-1), have specifically complementary linker sequences connected to their respective priming sequences via an adenine residue, and

(2) effecting a PCR reaction using a polymerase which adds a 3' adenine overhang to the end of an extended strand